



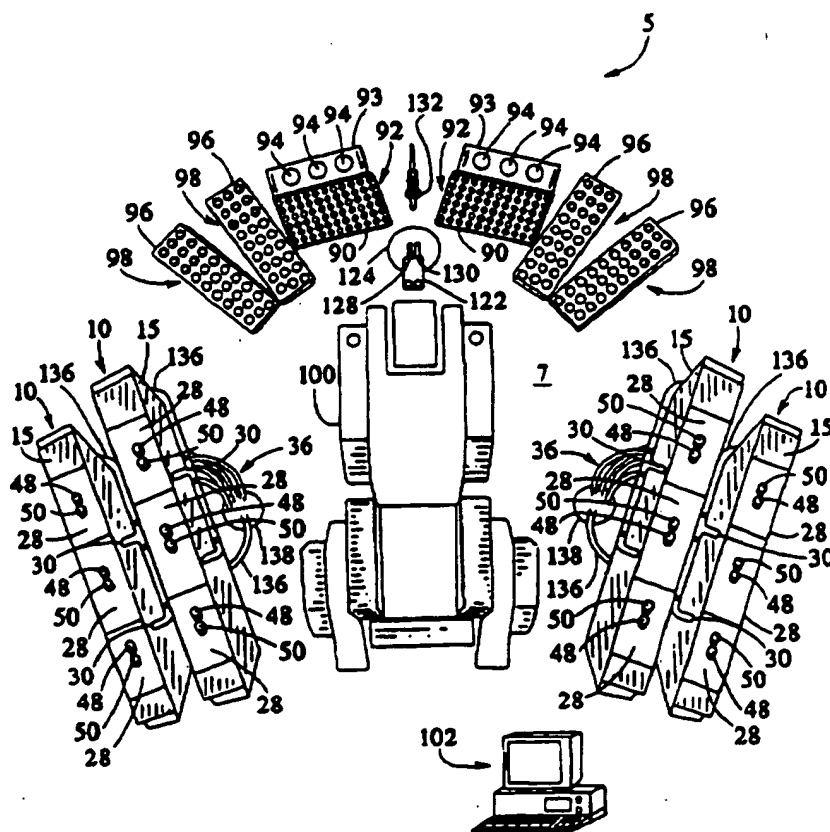
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(54) Title: **AUTOMATED PERMEABILITY ANALYSIS SYSTEM**

## (57) Abstract

The present invention relates to an automated permeability analysis system that increases the capacity, precision, accuracy and reliability of *in vitro* drug candidate permeability studies. The inventive permeability analysis system establishes a working environment around which a robotic arm may maneuver to enable a computer to establish and carry out a multitude of simultaneous drug transport experiments, with minimal test operator involvement. The system thereby may be used on a relatively large scale to easily and accurately investigate mechanisms of drug transport across a variety of cell membranes and tissues that act as barriers to drug absorption. In particular, the system may be used with monolayers of cultured epithelial cells.



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## AUTOMATED PERMEABILITY ANALYSIS SYSTEM

**BACKGROUND OF THE INVENTION**

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The present invention relates to a system for conducting drug candidate permeability studies, and, more particularly, a system for investigating the transport characteristics of drug candidates using *in vitro* models.

10 Cultured cell systems have been used in the past to study drug transport across specific biological barriers. Human colon cancer cells (CaCO-2), for example, have been cultured on filters and used with test chambers to create an *in vitro* model to study intestinal absorption of particular drug candidates. Drug candidates such as aspirin, acetaminophen, cimetidine, ranitidine, and nizatidine, for example, have all been studied in such models. Other types of absorption kinetic studies, such as buccal, sublingual, and other epithelial surfaces for  
15 example, are conducted in a similar manner.

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Higuchi, W.I., *Quantitative Mechanistic Studies in Simultaneous Fluid Flow and Intestinal Absorption Using Steroids as Model Solutes*, INT. J. PHARM, 4 (1980) 249-62; Kramer, C.Y., *Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications*, BIOMETRICS 12 (1956) 309-10; Leuenberger, H., Buchman, S., Reinke, C. and Schmid, B., *An In Vitro Absorption Model System Based on Cell Monolayers*, In Wilson, G., Illum, L., Davis, S.S. and Zweibaum, A. (Eds), Pharmaceutical Applications of Cell and Tissue Culture to Drug Transport, NATO ASI Series, Plenum, New York (1991) pp. 121-139; Ma, T.Y., Dyer, D.L. and Said, H.M., *Human Intestinal Cell Line Caco-2; A useful Model for Studying Cellular and Molecular Regulation of Biotin Uptake*, BIOCHIM BIOPHYS ACTA, 1189 (1994) 81-88; Shah P.K., Hidalgo, I.J. and Borchardt, K.L., *A simple Diffusion Device to Study Transport Across Cells Cultured on Microporous Membranes*, INT. J. PHARM, 63 (1990) 281-83; Strocchi, A. and Levitt, M.D., *A Reappraisal of the Magnitude and Implication of the Intestinal Unstirred Layer*, GASTROENTEROLOGY 101 (1991) 843-847; Thwaites, D.T., Brown, C.D.A., Hirst, B.H. and Simmons, N.L., *H + Coupled Dipeptide (glycylsarcosine) Transport Across Apical and Basal Borders of Human Intestinal Caco-2 Cell Monolayers Display Distinctive Characteristics*, BIOCHIM BIOPHYS ACTA, 1151 (1993) 237-45; Winnie, D., *Unstirred Layer as a Diffusion Barrier In Vitro and In Vivo*, In Skadhauge, E. and Heinize, K. (Eds), Intestinal Absorption and Secretion, MTP Press, Lancaster (1984) pp. 21-38; and Zheng, L., chen, J., Zhu, Y., Yang, H., Elmquist, W. and Hu M., *Comparison of the Transport Characteristics of D- and L-methionine in a Human Intestinal Epithelial Model (Caco-2) and in a Perfused Rat Intestinal Model*, PHARM RES., 11 (1994) 1771-76; the disclosures of which are incorporated herein by reference.

All such drug candidate transport studies typically are conducted in a manual fashion, whereby the substantial involvement of a test operator is required to ensure that the experiment is established and carried out in a timely and accurate manner. The experiments often prove to be quite labor intensive and time consuming in this regard. Moreover, such demands on the test operator escalate significantly when two or more drug transport experiments are being conducted simultaneously using multiple test chambers. The ever increasing need to rapidly and accurately assess the *in vitro* permeability of a series of compounds (or analogs within a series), however, demands a reliable, accurate, and high volume screening procedure.

It is therefore an object to the present invention to provide a system that significantly reduces the demands imposed on a test operator.

Another object of the present invention is to provide a system that increased the capacity, accuracy, and precision of drug candidate permeability studies.

5 It is also an object of the present invention to provide a system that can more quickly generate a larger amount of drug candidate permeability data to expedite drug candidate screening procedures.

Other objects of the present invention will be apparent by the following description.

### **SUMMARY OF THE INVENTION**

The automated permeability analysis system of the present invention meets these and other objectives, thereby providing a variety of benefits over earlier transport study methods and systems.

- 5           The inventive permeability analysis system establishes a working environment around which a robotic arm may maneuver to enable a computer to establish and carry out a multitude of simultaneous drug transport experiments, with minimal test operator involvement. The system thereby may be used on a relatively large scale to easily and accurately investigate mechanisms of drug transport across a variety of cell membranes and
- 10           tissues that act as barriers to drug absorption. The system can also support an SAR for co-optimization of drug activity and drug bioavailability. Moreover, the system similarly may be used as a screen for oral bioavailability in support of combinatorial chemistry approaches, insofar as there exists the potential for relative measurements of transport rates from stoichiometric mixtures of related compounds.

**BRIEF DESCRIPTION OF THE DRAWING**

A preferred embodiment of the present invention is described herein with reference to the drawing, wherein:

FIGURE 1 is a plan view of various table-top components of the preferred automated permeability analysis system, including a robotic arm at the center of the table;

FIGURE 2 is a front view of the robotic hand that is attached as a part of the robotic arm shown in Figure 1;

FIGURE 3 is a bottom view of the robotic hand that is shown in Figure 2 and attached as a part of the robotic arm shown in Figure 1;

FIGURE 4 is a schematic representation of additional components of the present invention located below the work table top;

FIGURE 5 is a cross-sectional schematic view of the preferred diffusion chamber that is used as a part of the present invention;

FIGURE 6 is an elevational view through the diffusion chamber shown in Figure 5 from the donor side, with the donor side end cap removed;

FIGURE 7 is a side view of the preferred cell culture insert loading tool that is used as a part of the present invention to insert and remove filters from the diffusion chamber shown in Figures 5 and 6;

FIGURE 8 is a perspective view of the insert loading tool shown in Figure 7;

FIGURE 9 is an elevational view of one chamber bank, having three diffusion chambers positioned by cradles within a water trough above three magnetic stirring devices;

FIGURE 10 is a perspective view of one of six cradles that are used in a chamber bank to properly position and maintain the diffusion chambers within the water trough; and

FIGURE 11(a) and 11(b) together present a flow chart diagram illustrating the operation of the automated test sequence of the present invention.



## **DESCRIPTION OF THE PREFERRED EMBODIMENT**

Referring now in detail to the various figures of the drawing, there is shown a preferred automated permeability analysis system of the present invention.

Figure 1, for example, is a plan view of various components of the preferred automated permeability analysis system 5

In certain embodiments, the permeability analysis system 5 is disposed within an enclosure capable of preventing light from contacting the samples that are to be analyzed. The enclosure must, of course, be large enough to allow the complete range of motion of robotic arm 100. The enclosure will preferably be used in situations where compounds to be analyzed are light sensitive.

Preferably, a 4' x 6' table top 7 acts as the work surface for the apparatus and experiments. Positioned on the table top surface are, for example, four chamber banks 10. As is best shown in Figure 9, each chamber bank 10 has a circulating water trough 15 formed from a polycarbonate material such as, for example, Lexan®. The troughs 15 are fed through water feed lines 136. In particular, each of the two lines 136 passes through an aperture 138 from beneath the surface of the table 7, and divides to serve a subset of the chamber banks, e.g., two chamber banks 10. Each chamber bank 10 includes two ports 30 through which water is received from a common branch of the divided feed line 136. The trough 15 is discharged through water falls 34 at either end of the chamber bank 10. The smaller the size of the water trough 15, the easier it generally is to control and maintain the temperature of the water.

Each chamber bank 10 also has a plurality of, e.g., three, diffusion chambers 20 situated within the water trough 15 above three magnetic stirring devices 25. The magnetic stirring devices 25 are configured such that the water trough 15 may rest on top of the series of three stirring devices 25. Situated within each stirring device 25 adjacent the bottom of the water trough 15 is a stirring motor having sufficient power to ensure proper mixing within the diffusion chamber 20.

Attached, e.g., glued to the inside surface of the water trough 15 are cradles 16 into which the diffusion chambers 20 are removably secured to ensure proper positioning of each chamber 20 in the chamber bank 10. Once the diffusion chambers 20 are properly positioned in the respective cradles 16, each station in the chamber bank 10 can be capped with a lid 28,

which is also formed a polycarbonate material such as from Lexan®, to reduce evaporation from the water trough 15.

The individual diffusion experiments are conducted within the diffusion chambers 20. The preferred diffusion chamber 20, which is best shown for example in Figures 5 and 6, is a unibody design constructed from Lexan®. The Lexan® material does not tend to bind to test compounds, is extremely rugged, and is rather easy to machine. Moreover, transparent Lexan® components, including the diffusion chamber 20, may be obtained by dipping the components in fumes of boiling chloroform. A transparent diffusion chamber permits observation of the experiment as the test progresses.

Figure 5 is a schematic cross-sectional view of the preferred diffusion chamber 20. The diffusion chamber 20 comprises a unibody portion 39 in which there is an apical or donor side 40 having a volume of approximately 14 mL, and a basolateral or receiver side 42 having a volume of approximately 13mL. During the experiment, the donor side 40 and the receiver side 42 are separated by a Millicell® PC or PCF culture plate insert device 44 having a 30 mm, 0.4  $\mu$  pore size filter 46. Sampling ports 48 and 50 fluidly communicate with the donor side 40 and the receiver side 42, respectively, and are of a sufficient height to extend through holes in the lids 28 so as to permit the robotic hand 104 to deliver and retrieve solutions from the diffusion chamber 20 without interfering with the lids 28. The sampling ports 48 and 50 are also beveled at their respective top edges 49 and 51 to cooperate with the pipette tips 92.

The ends of the diffusion chamber 20 are terminated with removable and interchangeable end caps 52 and 54, which each have a notch 17 to cooperate with a recess 18 of cradles 16 to secure the diffusion chamber 20 into position in the chamber bank 10. As is best shown in Figure 10, cradles 16 are glued on their sides and bottom to the water trough 15. For each diffusion chamber 20, two cradles 16 are secured in a spaced relationship within the water trough 15 to support the respective ends of the chamber 20. Each cradle 16 has two cut-out portions 19 to allow water to flow more easily past the cradle 16 within the water trough 16.

O-rings 56, 58 and 60 are used to seal the donor side 40 and the receiver side 42 of the diffusion chamber 20. Magnetic stir bars 62 and 64 rotate at a rate sufficient to adequately stir the chamber contents, e.g., 600 rpm, at the base of the diffusion chamber 20 in cooperation with the magnetic stirring devices 25 shown in Figure 9. The bottom exterior surface of the diffusion chamber 20 can be flattened, as is best shown for example in Figure 6, to help

stabilize the chamber 20 when resting on a flat surface, and to allow the stir bars 62 and 64 to more closely cooperate with the magnetic stirring devices 25 for greater stirring effect.

Figure 5 and 8 illustrate a loading tool 70 formed from Lexan® that is used to insert and remove culture plate insert devices 44, including the associated filters 46, from the diffusion chamber 20. In particular, the larger end 72 of the tool 70 fits within the receive side 42 of the chamber 20 and is used before the experiment to push the culture plate insert device 44 into position through the receiver side 42 of the chamber 20. To this end, the edge 74 of the tool 70 is configured to contact the bottom exterior surface of the culture plate insert device 44 at location 76 without disturbing the filter portion 46 of the device 44. Channels 78 and 79 extend through the tool 70 as shown in Figures 7 and 8, and allow air pressure to be released through the tool 70 as the culture plate insert device 44 is inserted into position. This helps prevent any damage or other disruption to the filter 46 and any cells thereon.

Once the experiment is complete, the small end 80 of the tool 70 is inserted through the donor side 40 of the chamber 20 and is used to push the culture plate insert device 44 out of the diffusion chamber 20 through the receiver side 42. The beveled edge 82 of the tool 70 is similarly configured to contact the bottom interior surface of the culture plate insert device 44 at location 84, again without disturbing the filter portion 46 of the device 44.

Referring once again to Figure 1, also situated on the table top are two pipet tip holder racks 90 to house up to a total of 144 pipette tips 92, two source racks 93 to house six source vials 94 for sample or test solutions, and four sample vial racks 93 to house up to a total of 132 sample vials 98 in which donor and receiver samplings are collected for up to 12 simultaneous transfer experiments. Sample vials 98 preferably are constructed from glass.

Each of the 12 columns of sample vials 98 shown in Figure 1 corresponds to one of the twelve diffusion experiments being conducted. Moreover, the dark-colored vials represent sample vials 98 in which samplings from the donor side 40 of the diffusion chambers 20 are preferably kept during the experiment, while the light-colored vials represent sample vials 98 in which samplings from the receiver side 42 are preferably kept. Each of the receiver sample vials includes a flat-bottomed glass insert so that the receiver sample sorted within the vial is easier to retrieve from the vial for analysis. The donor sample vials do not require an insert in the preferred embodiment because the more concentrated donor samples are diluted within the vial before analysis, making retrieval easier.

Bolted onto the center of the table is a Mitsubitshi Movemaster EX robotic arm 100, which has a range of movement around the surface of the table top of approximately 300 degrees. Computer 102 is programmed in a conventional manner, such as in GW BASIC, to control the various operations of the robotic arm 100. The robotic arm 100 is used during the experiment to transfer liquids as required by the experiment. Therefore, the source vials 94, the sample vials 98, the pipette tips 92, and the sampling ports 48 and 50 of all involved diffusion chambers 20 must be accessible to the robotic arm 100 during the experiment. To this end, the two chamber banks 10 immediately adjacent the robotic arm 100 are more elevated off the table top surface 7 than are the chamber banks 10 at the outer edges of the table.

A hand 104 is attached to the robotic arm 100 through a stainless steel circular disc block attachment piece 106, as is shown for example in Figures 2 and 3. The disc 106 is attached to the robotic arm 100 for example with screws that pass through holes 107 in the disc 106. The hand 104 itself includes a rectangular stainless steel block 108 in which there are bored three right-angled channels 110, 112 and 114 for the passage of air and/or liquids. Each of the three channels 110, 112 and 114 has a threaded opening at both the rear side and the bottom side of the stainless steel block 108. Attached to the bottom side of the block 108 are two stainless cannulae 116 and 118, which are in fluid communication with channels 110 and 114, respectively. The two cannulae 116 and 118 are used to deliver buffers as required for the experiment.

Also attached to the bottom of the block 108 is one stainless steel pipet tip receiver 120, which communicates with channel 112. The pipet tip receiver 120 can pick up a disposable plastic pipet tip 92 by entering the upper opening of the pipet tip 92 to the point where a frictional fit is created between the inside surface of the pipet tip 92 and the outside surface of the pipet tip receiver 120, as shown for example in Figure 2. The computer 102 controls the movements of the robotic arm 100 to ensure that the cannulae 116 and 118 of the robotic hand 104 do not obstruct or otherwise inhibit the pick-up or maneuvering of the pipette tip 92.

A pipet tip extractor 122 is secured to the table top 7 and disposed over a hole 124 that leads to a waste bucket 126 below the table surface. A pipette tip 92 is removed from the robotic hand 104 by placing the pipette tip 92 between the two lines 128 and 130 of the extractor 122, and then lifting up on the robotic arm 100. The frictional fit between the

pipette tip 92 and the pipette tip receiver 120 is thereby relieved, and the pipette tip 92 falls into the waste bucket 126 below the table surface. A sensor 132 is positioned adjacent the hole 124, and is used by computer 102 to verify that the robotic arm 100 has either successfully picked-up or successfully disposed of a pipette tip 92.

5 Since the robotic arm 100 must move throughout the experiment to a number of defined locations, most table top components described above are removably secured to the table top 7 by pins so as to ensure proper positioning of the various components on the work surface 7 relative to the robotic arm 100.

Referring now to figure 4, located on a shelf below the surface of the work table are  
10 two VanKel heater/circulator water pumps, 134, which maintain a 37°C water temperature and circulate the water to the water troughs 15 through circulating lines 136 that pass through apertures 138 in the table top. The 37°C water temperature simulates the 37°C temperature of the human body. Two water collector devices 140 receive the water returning from the troughs 15 through return lines 36. The two respective VanKel pumps 134 are fed from the  
15 bottom of the water collector devices 140 so as to avoid the introduction of air pockets into the water system.

Also situated under the table top are the controller 142 for the robotic arm 100, two switch controls 144 that each turn on and off a series of, for example, six magnetic stirring devices, 25, the waste bucket 126 to receive pipette tip waste, and a printer 146 that  
20 communicates with the computer 102 to print reports. A WTI smart switch 148, model SS-8B, acts as a communications hub for the computer 102 and various other components of the system, such as, for example, the robotic arm 100, the printer 146, and other components described below.

Two Hamilton syringe/dilutors 150 and 152 below the table top communicate through  
25 lines 154, 155, and 156 with the three ports 160, 162, and 164 at the back side of the robotic hand 104. In particular, syringe/dilutor 150 contains two 5 mL syringes. The first of the two 5 mL syringes is used to deliver a pH 6 transport buffer to port 160 of the robotic hand 104 through fluid line 154. The pH 6 transport buffer is maintained at 37°C in water bath 141 the second of the two 5 mL syringes is used to deliver a pH 7.4 transport buffer to port 164 of the  
30 robotic hand 104 through fluid line 156. The pH 7.4 transport buffer is also maintained at 37°C in water bath 141. Syringe/dilutor 152 has one 250 µL syringe that communicates through air line 155 with port 162 of the robotic hand 104, and is used to provide either an air

flow or a vacuum at the pipette tip receiver 120 as necessary. The computer 102 works through the smart switch 148 to activate the various syringes of the syringe/dilutors 150 and 152 at the appropriate times.

5 A programmable logic controller board 166 below the table top includes two output chips and one input chip. The two output chips of the controller board 166 work with the smart switch 148 to provide communication between the computer board 166 work with the smart switch 148 to provide communication between the computer 102 and two computer-controlled flow valves 168 and 170, which control fluid communication between the two syringes of the syringe/dilutor 150 and the respective ports on the robotic hand 104. The  
10 pipette sensor 132 on the table top also communicates with the computer 102 through the input chip of the computer board 166.

The preferred diffusion experiment strives to mimic the drug absorption process that occurs, for example, in the small intestine. Inside the small intestine, or lumen, is a tubular structure that is covered with polarized epithelial cells. Successful oral drug candidates pass  
15 from the lumen, across the epithelial cell barrier, into the blood stream.

Accordingly, for the preferred experiment human colon cancer cells (CaCO-2) are cultured on the filter portions 46 of the culture plate insert devices 44. The cancer cells may be obtained, for example, from the ATCC (American Type Culture Collection, Rockville, MD). It will be apparent to those of skill in the art that alternative cell lines may instead be  
20 used to perform other types of studies. Endothelial cells, for example, may be used to study the transport of drug candidates across the blood/brain barrier.

The microporous culture plate insert devices 44 may be obtained, for example, from the Millipore Corporation. The culture plate insert devices 44, with cells grown thereon, are first subjected to an electrical resistance barrier integrity check, and then manually loaded  
25 with the tool 70 into the various diffusion chambers 20. The diffusion chambers 20 are then placed at the various stations in the water troughs 15 above the magnetic stirring devices 25.

Referring now to the flow chart diagram presented by Figures 11(a) and 11(b), it is shown that the automated test sequence begins at block 174 where the computer system 102 and all associated communication links are initialized. Block 175-179 show that certain  
30 parameters may be inputted into the computer system 102, including total duration of the experiment, the amount of sample/test solution to be withdrawn from which source vial, an identification of which stations are included in the overall experiment, and whether the fluid

lines 154 and 156 should either be flushed before the experiment commences, or washed at the conclusion of the experiment. Block 180 shows that fluid lines 154 and 156 are first flushed with the respective buffer solutions if previously requested in connection with block 179.

5           Once the system has been initialized and appropriately configured, the donor side 40 and receiver side 42 of each diffusion chamber 20 are filled with pH 6 and pH 7.4 transport buffers, respectively, as is represented by block 181 of the flow chart diagram. The pH of the lumen, where most of the absorption takes place, is 6, and the pH of blood is 7.4. The transport buffers are placed by the cannulae 116 and 118 of the robotic hand 104 into the  
10           diffusion chambers 20 through the two sampling ports 48 and 50. The cannulae 116 and 118 are therefore preferably positioned on the robotic hand 104 so as to permit simultaneous transfer of the transport buffers into the respective sides of the diffusion chamber 20. Simultaneous introduction of the transport buffers into the diffusion chamber 20 tends to equalize the pressures within the chamber 20, thereby helping to prevent any disruption to the  
15           filter 46.

          Referring now to blocks 182-186, each station is first set up by securing a clean pipette tip 92 on the robotic hand 104, and drawing up to 400  $\mu$ L of a test compound from a source vial 94 into the pipette tip 92 by a vacuum generated by the syringe/dilutor 152. The test compound within the pipette tip 92 is then delivered to the donor side 40 at the particular test  
20           station, and the delivery time is recorded. Three initial donor samples are taken with a clean pipette tip 92, the pipette tip 92 being first wetted before sampling. The three initial donor samples are delivered to three separate sample vials 98, each pipette tip 92 being touched off at the end of each delivery. The computer 102 then calculates, based upon the test compound delivery time, when samples should be taken from the receiver side 442 during the course of  
25           the experiment.

          The preferred sample times for a two-hour experiment, for example, would be at 30, 60, 90, and 120 minutes as measured from the original test compound delivery time. For a one-hour experiment, on the other hand, the preferred sampling times would be at 15, 30, 45, and 60 minutes as measured from the original test compound delivery time.

30           The entire process is repeated until the donor side 40 at each test station contains the appropriate test compound for the experiment. During this time it may become necessary to

take a receiver sample from one of the stations, depending upon the time tables calculated in connection with block 186.

Blocks 182 and 187-192 of Figure 11(a) relate to the measurement, as a function of time, of how much of the test compound in each diffusion chamber 20 is transported (i.e., permeates) from the donor side 40 through the cell barrier into the buffer on the receiver side 42. In the preferred procedure, such measurements are made by using clean pipette tips 92 to periodically sample approximately 200  $\mu$ L of the solution on the receiver side 42 during the course of a study that may last, for example, two hours. The time is recorded each time a sample is taken. The resulting samples are stored in individual clean sample vials 98, each pipette tip 92 being touched off at the end of each delivery. After a sample is taken, the volume of solution withdrawn from the receiver side 42 is replaced with pH 7.4 buffer so as to maintain a constant volume. Once it is determined that the last receiver sample has been taken for a particular station, two final donor samples are individually taken with clean, wetted pipette tips 92, and delivered to clean sample vials 98, as is shown for example by blocks 190 and 191. Again, the pipette tips 92 are each touched off after each delivery.

The computer 102 is configured to carry out all of these delivery and sampling procedures at the appropriate time intervals, as dictated by the time tables established in connection with block 186.

Referring now to block 192 of Figure 11(a), the sampling routines conclude once it is determined that all test compound experiments have been completed at all stations. The samples placed in the sample vials 98 during the course of the experiment may then be analyzed to determine the permeability of test compounds. Such analysis may include, for example, the use of high pressure liquid chromatography (HPLC) with fluorescence detection. Spreadsheet software programs may be used as a part of the data analysis, and may include an identification of the total length of the experiment, the sample amount, the source vial, and the particular sampling times and results for each station involved in the experiment.

The preferred test procedure then continues for the purpose of determining whether any of the stations experienced an unacceptable amount of leakage during the course of the experiment. Such leakage may result from, for example, a filter 46 that was damaged when the culture plate insert device 44 was installed in the diffusion chamber 20. The check is preferably made using the leakage marker check procedure that is shown for example in Figure 11(b) and described below.



The source vials 94 of test compound are first placed with source vials 94 containing a solution of Molecular Probes SR101 leakage marker. Referring not to Figure 11(b), the leakage marker check is set up by using clean pipette tips 92 to add up to 400  $\mu$ L of leakage marker solution to the donor side 40 of each diffusion chamber 20 used in the test compound experiment. For each diffusion chamber 20, the leakage marker solution is preferably obtained from the same source vial 94 position as was the original test compound. The delivery time of the leakage marker solution is recorded, and the computer 102 calculates, based upon the delivery time, when samples should be taken from the diffusion chamber 20 during the course of the leakage marker test.

Blocks 196-198 of Figure 11(b) show that samples are taken at the appropriate time intervals from both the receiver side 42 and the donor side 40. In particular, the preferred sampling occurs between 20 and 30 minutes after delivery of the leakage marker solution to the donor side 40, whereby clean pipette tips 92 are used to take on 200  $\mu$ L sample from the receiver side 42 and on 100  $\mu$ L sample from the donor side 40 at each station. Individual samples are deposited in individual clean sample vials 98. Again, the pipette tips 92 are wetted and touched off during this procedure.

The purpose of the sampling is to determine how much of the leakage marker solution passes from the donor side 40 to the receiver side 42 at each station during the test period. If subsequent analysis to the receiver sampling evidences an unacceptably high level of transferred leakage marker solution for one or more diffusion chambers 20, then the test compound permeability results corresponding to these particular diffusion chambers 20 can be either carefully scrutinized or disregarded altogether.

Once again, the computer 102 is configured to carry out all of these delivery and sampling procedure at the appropriate time intervals, as dictated by the time tables established in connection with block 195.

At the conclusion of the leakage marker test, the two syringes of syringe/dilutor 150, as well as lines 154 and 156, are automatically washed with clean water contained in the rinse flask 172 if such an option was originally selected in connection with block 179. The culture plate insert devices 44 are also manually unloaded from the diffusion chambers 20. The diffusion chambers 20 clean-up rather quickly and easily as a result of their unique design.

It will be apparent to those of ordinary skill in the art that the automated permeability analysis system disclosed and describe herein can be modified without departing from the true spirit and scope of the invention.

**WHAT IS CLAIMED:**

1. A device for determining drug permeability characteristics across a biological barrier comprising

5 (a) a plurality of diffusion chambers, the diffusion chambers having an apical portion adjacent a basolateral portion and a culture plate removably disposed between the apical and basolateral portions; and

(b) an automated means for providing compounds to the apical portion and obtaining samples from the basolateral portion.

10

2. A device according to Claim 1, further comprising a means for maintaining isothermal conditions about the diffusion chambers.

15

3. A device according to Claim 1, wherein the culture plate includes a filter portion.

4. A device according to Claim 3, wherein cells are disposed on the filter portion.

20

5. A device according to Claim 1, wherein the automated means for providing compounds comprises a computer controlled means for transferring liquid samples.

6. A device according to Claim 4, wherein the computer controlled means for transferring samples comprises a robotic arm.

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7. A device according to Claim 4, wherein the robotic arm includes a hand having at least one port for the passage of gas or fluid.

8. A device according to Claim 1, wherein a plurality of the the diffusion chambers are removably mounted in a chamber bank.

30

9. A device according to Claim 1, wherein the chamber banks are formed from polycarbonate.

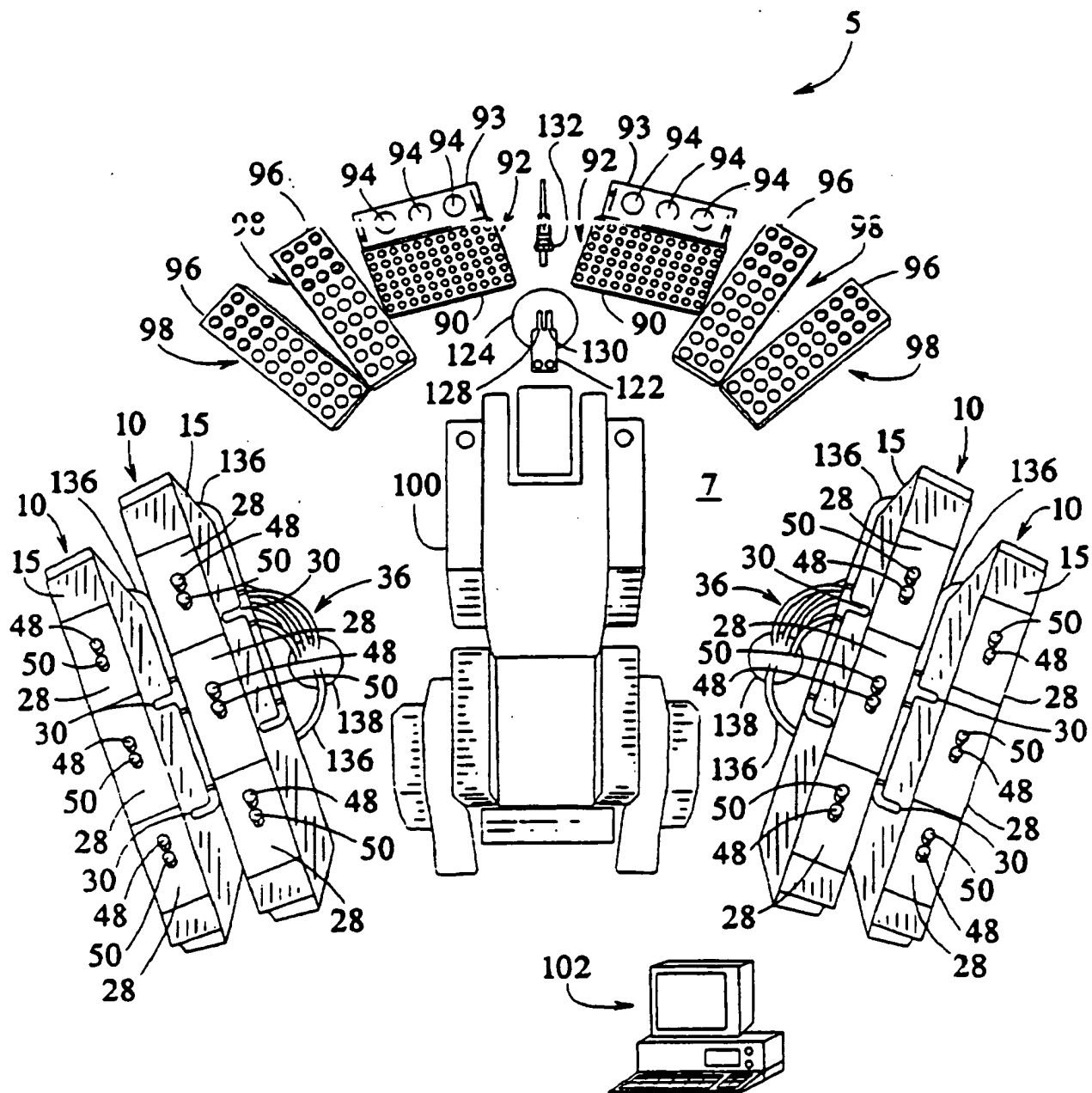
10. A device according to Claim 1, wherein a magnetic stirring device is disposed beneath at least one diffusion chamber.

5 11. A device according to Claim 1, wherein the means for maintaining isothermal conditions comprises a water troughs provided in the chamber banks.

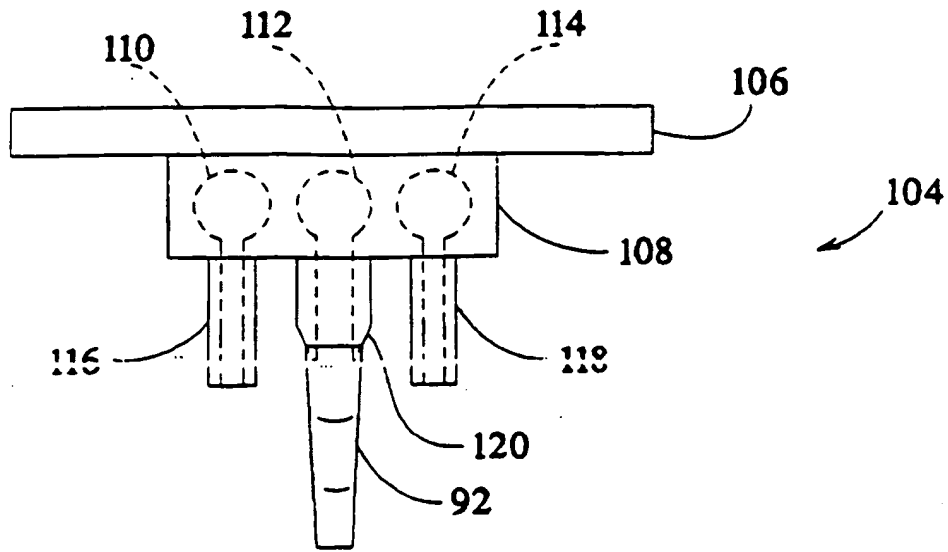
12. A device according to Claim 1, wherein the device is situated on a table surface.

10 13. A device according to Claim 6, wherein the robotic arm has a 300° range of motion about the table surface.

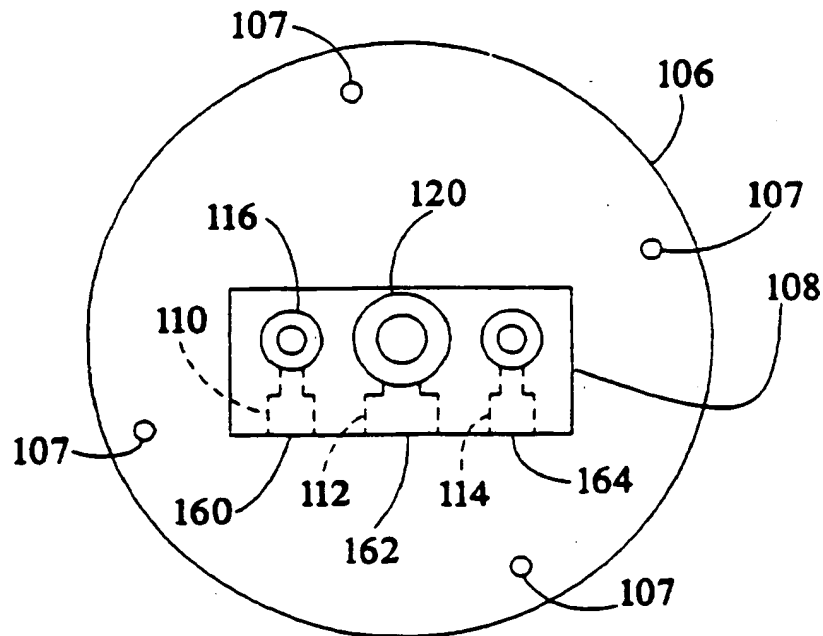
15 14. A device according to Claim 1, wherein the diffusion chambers are formed from polycarbonate.

FIG. 1

**FIG. 2**



**FIG. 3**



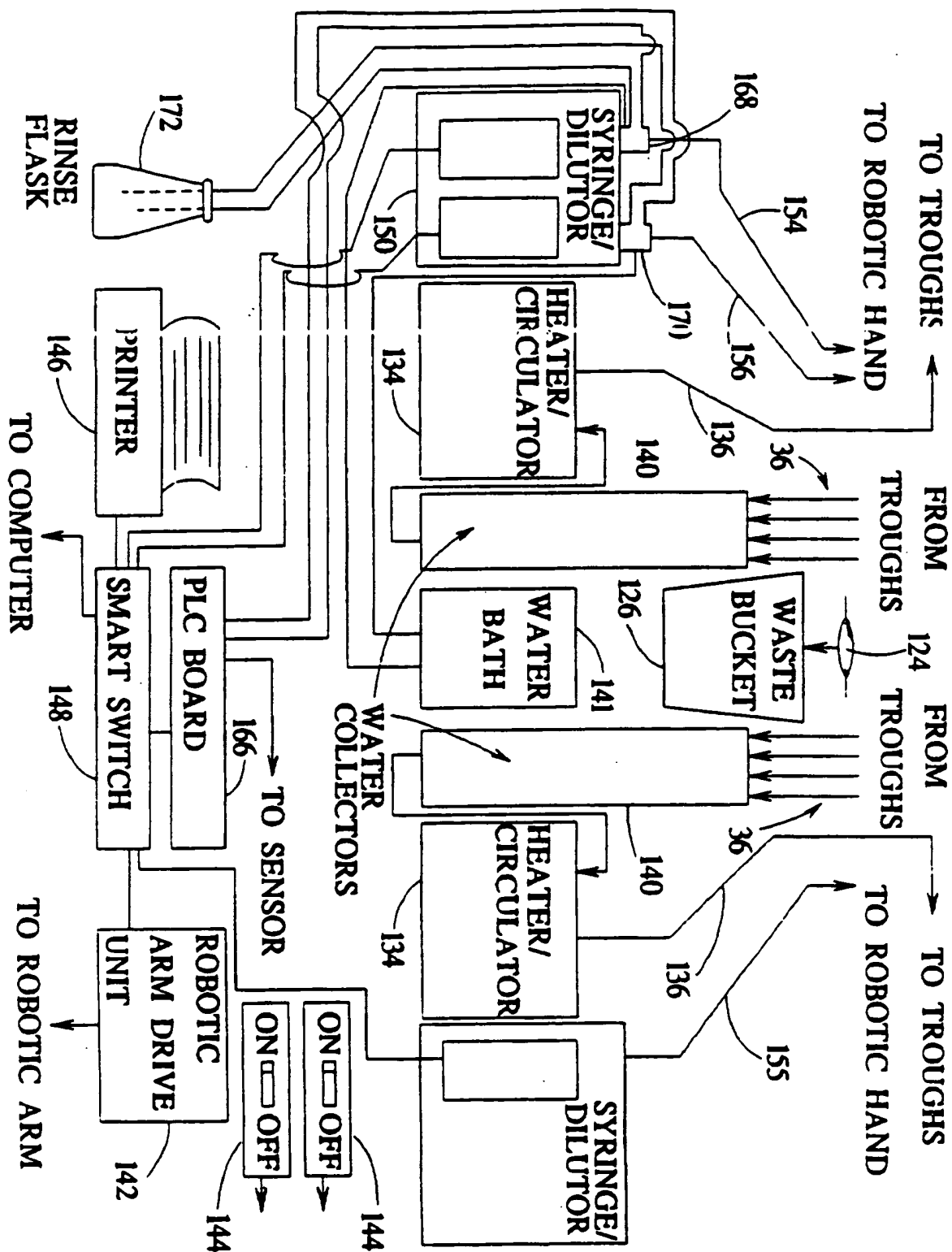
**FIG. 4**

FIG. 5

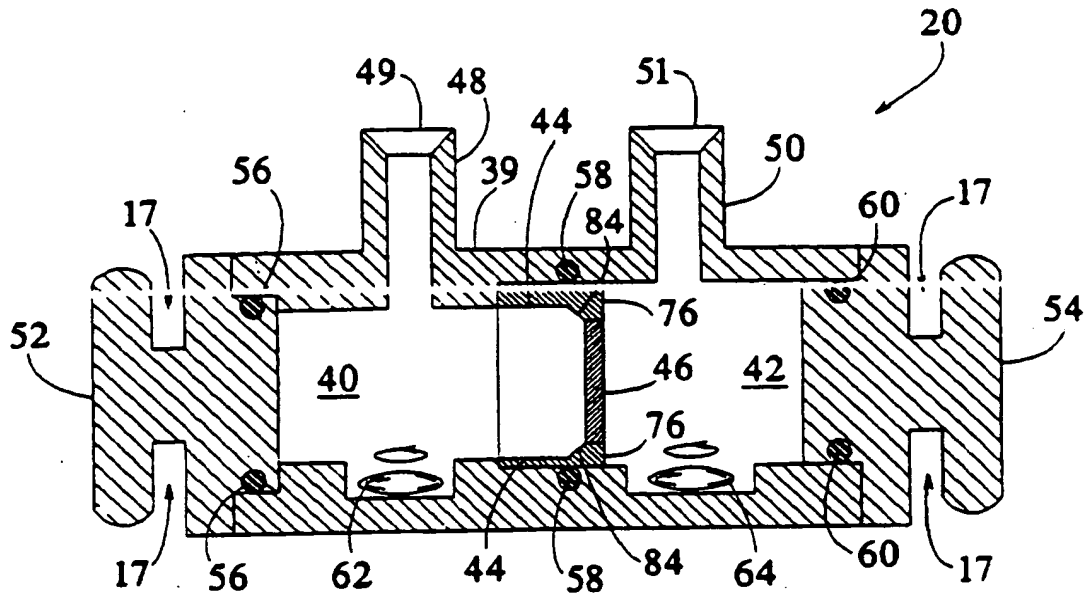


FIG. 6

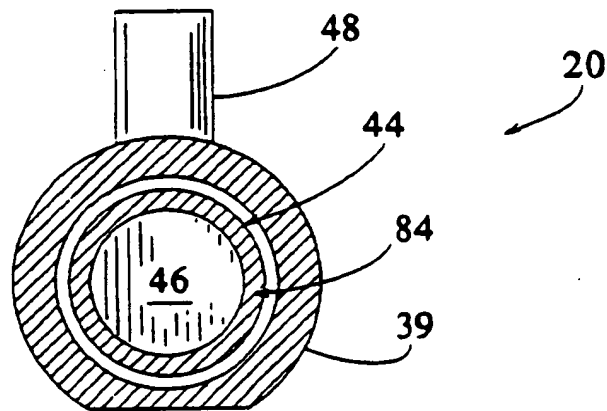
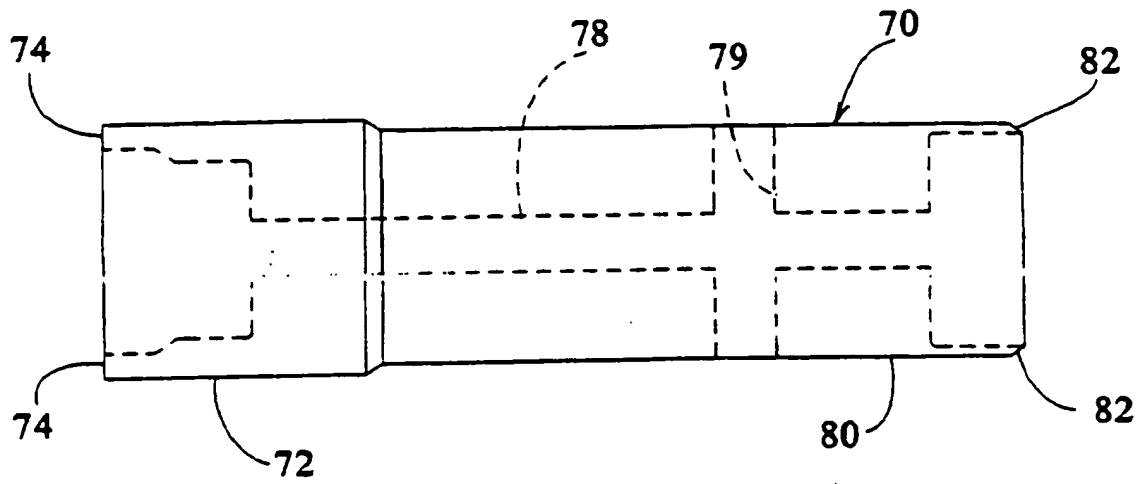
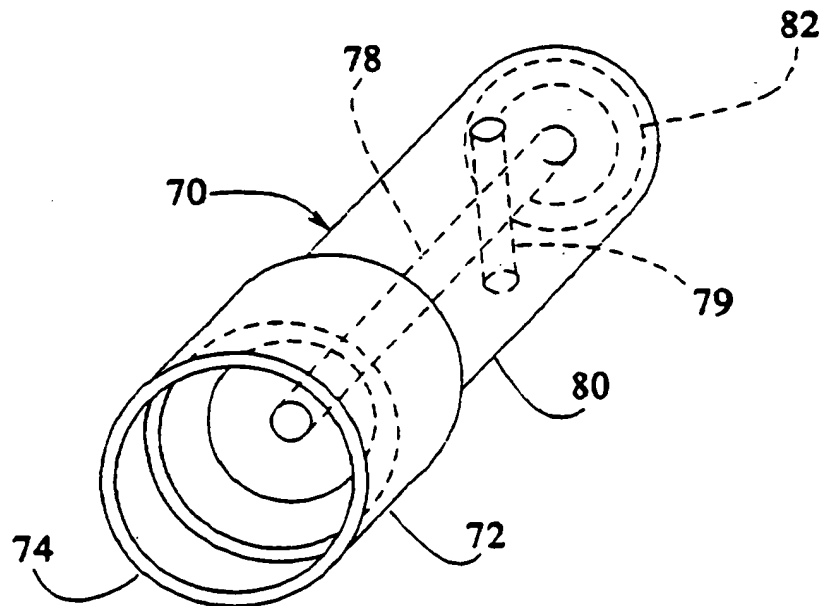




FIG. 7FIG. 8

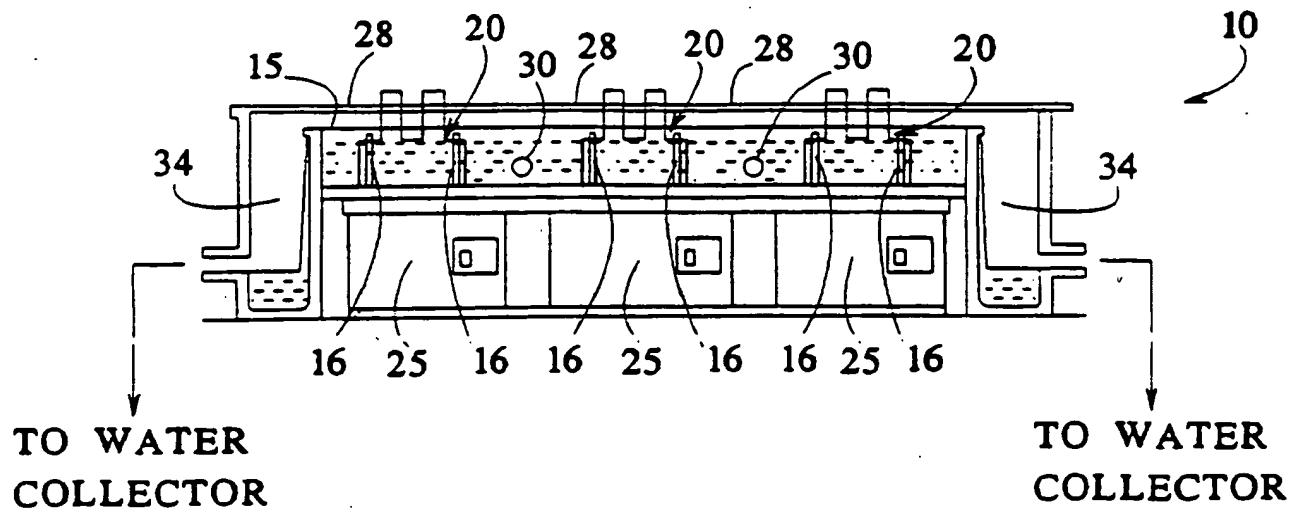
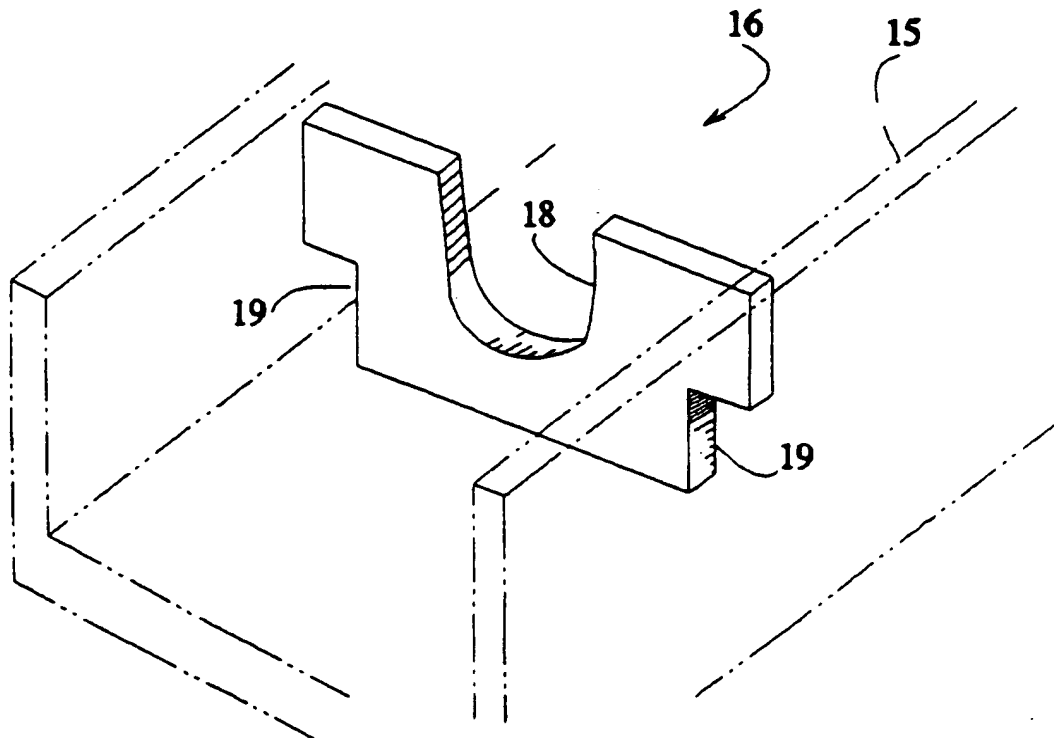
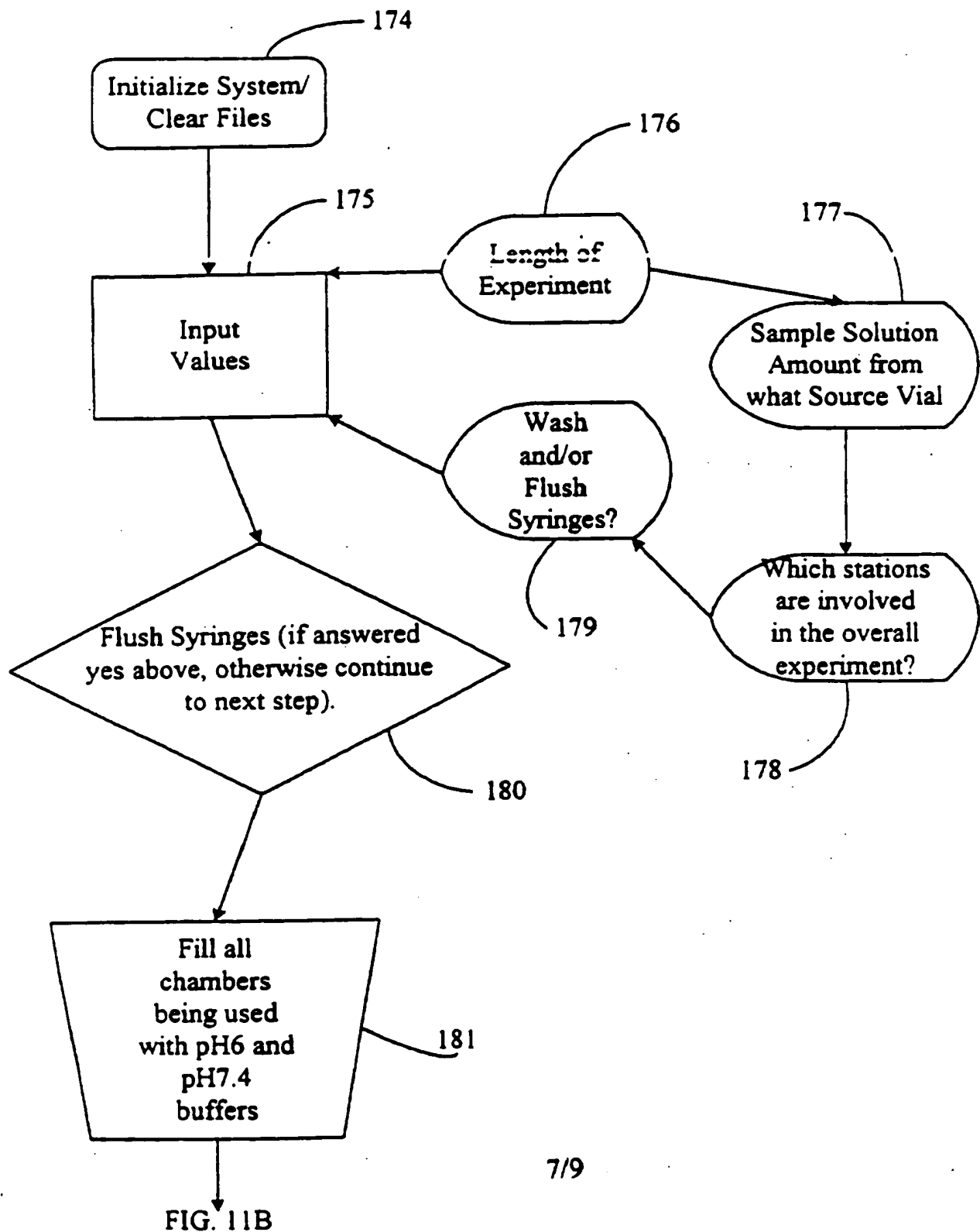
**FIG. 9****FIG. 10**

FIG. 11A

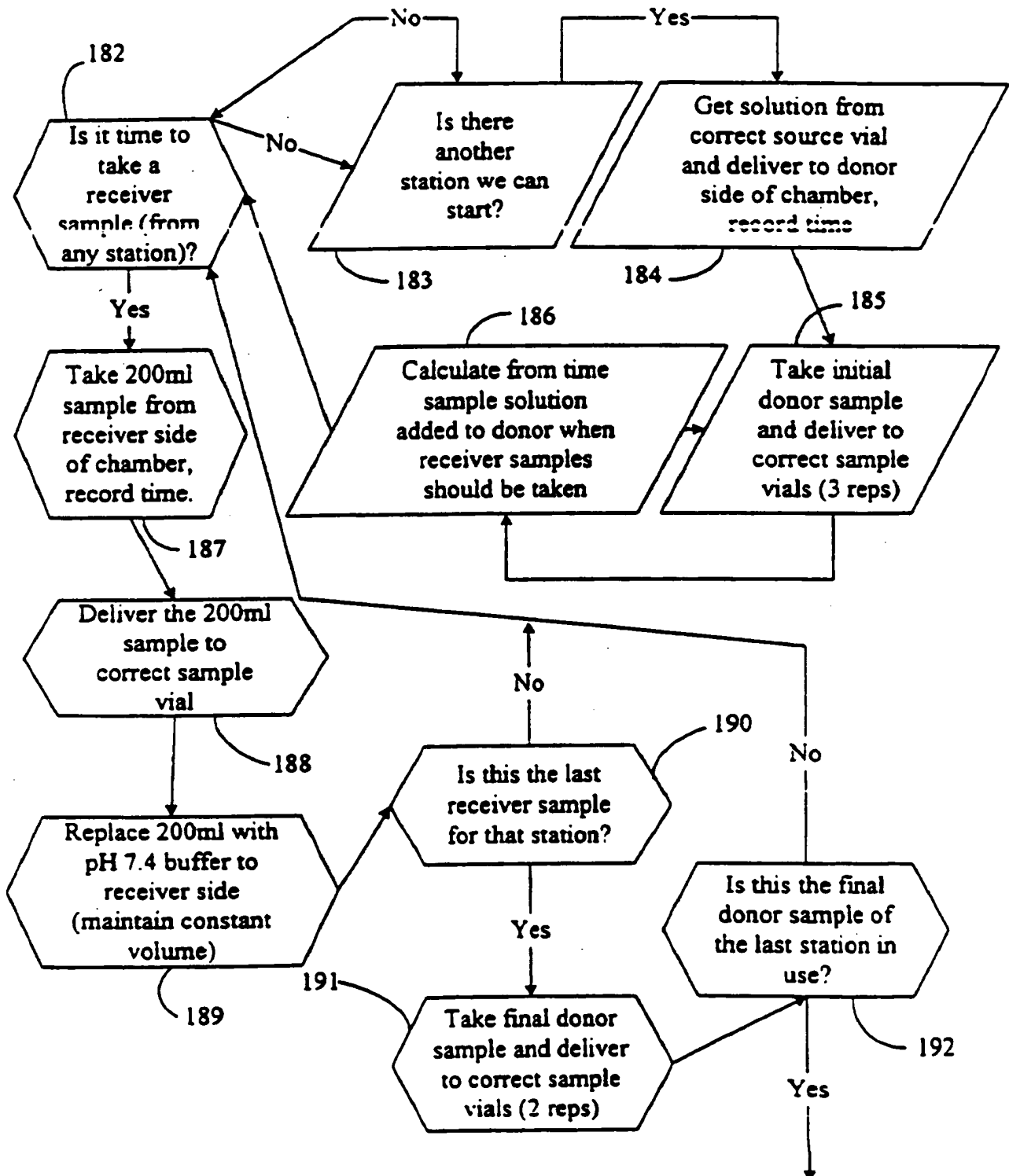
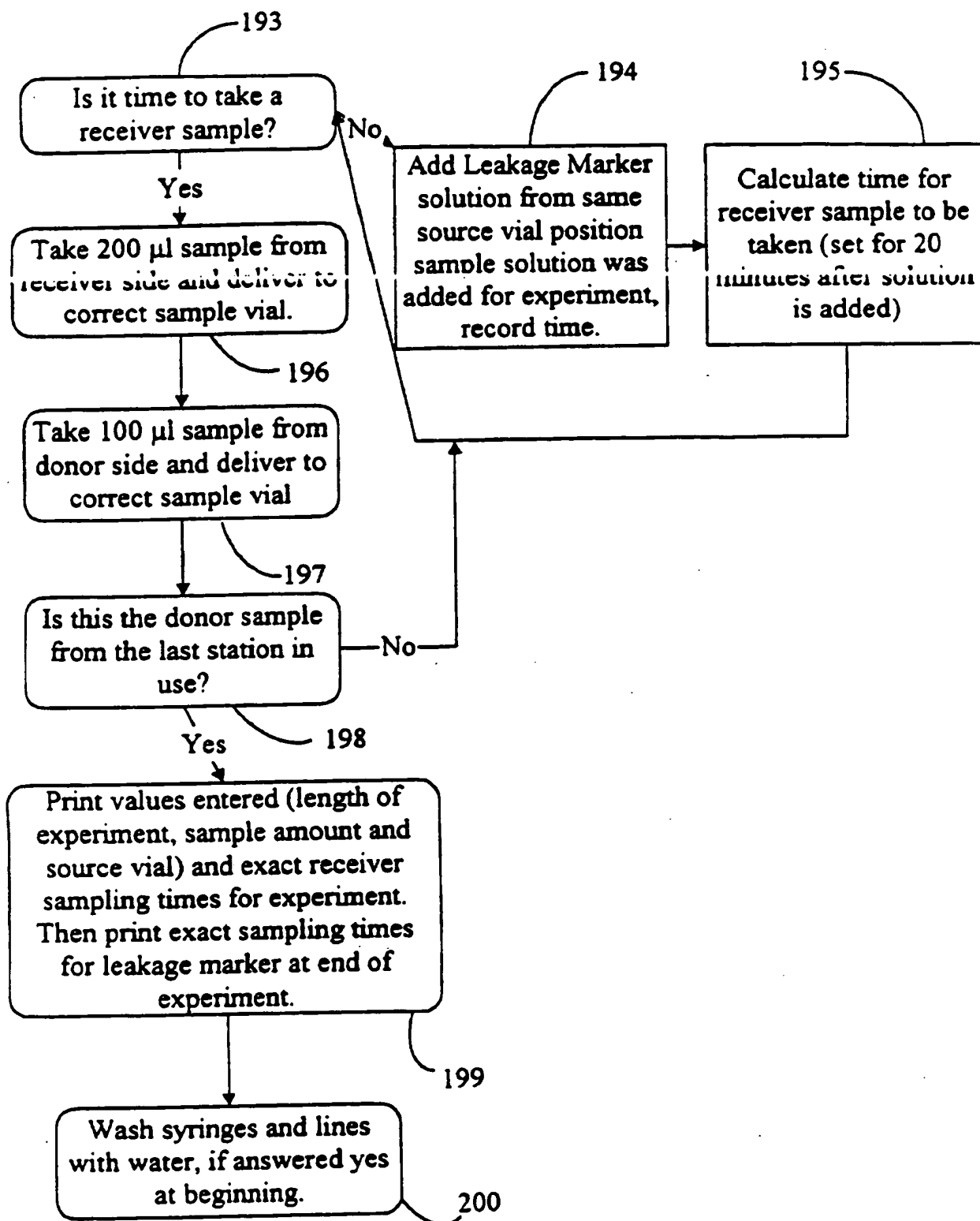
*FIG. 11B*

FIG. 11C

# INTERNATIONAL SEARCH REPORT

National Application No.

PCT/US 96/17339

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 6 G01N13/00 G01N33/15

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 83 03901 A (PHARMACONTROL CORP) 10 November 1983	1,3-5,8
A	see page 10, line 25 - page 12, line 32  see page 15, line 24 - page 19, line 3 see page 30, line 32 - page 32, line 11 see page 33, line 13 - page 36, line 5; figure 10	2,6,7,9, 10,12
A	--- PHARMACEUTICAL RESEARCH, vol. 8, no. 2, 1991, US, pages 222-227, XP000613852 I.J. HIDALGO ET AL.: "CHARACTERIZATION OF THE UNSTIRRED WATER LAYER IN Caco-2 CELL MONOLAYERS USING A NOVEL DIFFUSION APPARATUS" cited in the application see page 222 - page 223; figure 1 --- -/--	1-4,8,9, 11,14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

**\* Special categories of cited documents:**

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Date of the actual completion of the international search

7 February 1997

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/17339

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 183 760 A (SWEETANA STEPHANIE A ET AL) 2 February 1993 see column 4, line 25 - column 7, line 22; figures	1-4,8,9, 11,14
P,X	--- US 5 490 415 A (MAK VIVIEN H W ET AL) 13 February 1996 see column 5, line 55 - column 7, line 63; figures	1-6,8,10
A	--- INTERNATIONAL LABORATORY, vol. 16, no. 9, November 1986, FAIRFIELD CT US, page 56,58,60 XP002024776 J.R.STRIMAITIS: "Pharmaceutical quality control using laboratory robotics" see the whole document	1,6,12, 13
A	--- INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 63, no. 3, 30 September 1990, AMSTERDAM, NL, pages 281-283, XP000613859 P.K. SHAH ET AL.: "A simple diffusion device to study transport across cells cultured on microporous membranes" cited in the application see page 282; figure 1 -----	1-4,10

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 96/17339

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-8303901	10-11-83	US-A- 4578244 AU-A- 1608983 CA-A- 1210252 EP-A- 0106892	25-03-86 21-11-83 26-08-86 02-05-84
US-A-5183760	02-02-93	NONE	
US-A-5490415	13-02-96	NONE	